A *Chilo* Iridescent Virus (CIV) from the Rice
Stem Borer, *Chilo suppressalis* WALKER
(Lepidoptera : Pyralidae)

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(Received March 4, 1966)

A new iridescent virus obtained from two larvae of the rice stem borer, *Chilo suppressalis* WALKER, was described and named *Chilo* iridescent virus (CIV). It seemed to be the first finding of this kind of virus in a lepidopterous insect collected in nature. The virus particle appeared hexagonal in outline and the average diameter was about 160 m μ . Although it showed morphological similarity to TIV, little serological relationship between CIV and TIV was so far observed. The larvae of the rice stem borer were readily infected with CIV by intrahemocoelic inoculation or by feeding.

INTRODUCTION

In early summer of 1964, we received two dead larvae of the rice stem borer, *Chilo suppressalis* WALKER from Dr. K. KOJIMA of the Towa Agric. Chem. Co. These larvae were originally collected by Mr. I. TATEISHI¹ of the Fukuoka Agricultural Experiment Station in the Tsukushino-machi paddy field, Fukuoka Prefecture, Kyushu, Japan. The infected larvae appeared brownish black in colour when they reached our laboratory. Because the tissues of the insects were too desiccated to examine, an attempt was made to multiply the disease agent in rice stem borer larvae. Fortunately this was successfully performed, and the characteristics of this disease were to some extent clarified.

MATERIALS AND METHODS

The original dead larvae were triturated in distilled water, and the resulting suspension was added to the artificial diet on which healthy 3rd instar larvae of the rice stem borer were growing. After the oral inoculation the larvae were kept at 25°C. Twelve days later some larvae became milky white, and 9 out of 55 larvae were clearly infected within 20 days. The colouration of infected larvae probably varied according to the degree of infectivity, but heavily infected larvae looked bluish. The remarkable fact was that the diseased larvae could survive for a considerably long time.

Fat tissues of infected larvae were fixed in osmium tetroxide solution buffered

¹ Mr. I. TATEISHI found for the first time this type of diseased larva at Dazaifu, Fukuoka Prefecture, Nov. 18, 1960 (Personal communication).

at pH 7.2 and embedded in Epon 812 by LUFT's method: ultrathin sections were stained with uranyl acetate and lead acetate and examined with the electron microscope (Hitachi HU-11).

On the other hand, whole infected larvae were triturated in 1/40M Tris buffer (at pH 7.2) and were centrifuged for 15 minutes at about 3,000 r.p.m. The supernatant was centrifuged for 30 minutes at about 5,000 r.p.m., and then was centrifuged for 30 minutes at about 10,000 r.p.m. Such centrifugation was repeated three times, and the resulting iridescent pellet was suspended in Tris buffer and the suspension was named fraction A.

EXPERIMENTAL RESULTS

Ultrathin sections of fat cells of the infected larvae showed many hexagonally shaped non-inclusion virus particles in the cytoplasm, but no particles were found in the nucleus (Fig. I a, b).

Fig. Ia. Electron micrograph of *Chilo* iridescent virus particles in the fat body cell of infected rice stem borer larva. E: empty particles; N: nucleus; V: virus particles. Fig. Ib. Highly magnified CIV showing electron dense cores. c: core; s: shell of virus particle.

An electron micrograph of the virus obtained from fraction A indicated virus particles of about $160\text{ m}\mu$ in diameter which had envelopes on the outside (Fig. IIa, b). The virus particles were also observed with negative staining. As shown in Fig. IIb, inside of the envelope of the empty particle there was a thin shell which had no definite structure. The central part of the virus particle was generally

Fig. II a. Electron micrograph of CIV in fraction A shadowed with chromium. Fig. II b. CIV negatively stained with phosphotungstate. ev: envelope; s: shell of empty particle.

electron dense and seemed to be the core of the virus.

It may be added that a small amount of fraction A injected into diapausing larvae induced 100 per cent infection within a week at 25°C.

This virus (CIV) is morphologically similar to TIV, SIV, and MIV except in diameter (XEROS, 1954; STEINHAUS and LEUTENEGGER, 1963; CLARK *et al.*, 1965). However, a serological relationship between TIV and CIV seems to be negative (unpublished data), and no relationship was obtained between SIV and CIV (DAY, personal communication²).

To our knowledge this is the first iridescent virus obtained from a lepidopterous insect in nature, and it will be named *Chilo* iridescent virus (CIV).

ACKNOWLEDGEMENTS

We wish to thank Prof. H. ARUGA and Dr. T. HUKUHARA, Faculty of Agriculture, University of Tokyo, for providing the TIV suspension used in this study. Thanks are also due to Dr. J. MITSUHASHI and Mr. S. KAMANO, Division of Entomology, National Institute of Agricultural Sciences, for advice in the preparation of the experiment.

² According to Dr. M. F. DAY of the C. S. I. R. O. in Canberra, serological relationship between SIV and CIV by gel diffusion techniques has been completely negative (personal communication).

REFERENCES

- CLARK, T. B., W. R. KELLEN and P. T. M. LUM (1965) A iridescent virus (MIV) from *Aedes taeniorhynchus* (WIEDEMANN). *J. Invert. Pathol.* 7 : 519-521.
- STEINHAUS, E. A. and R. LEUTENEGGER (1963) Icosahedral virus from a scarab (*Sericesthis*). *J. Insect Pathol.* 5 : 266-270.
- XEROS, N. (1954) A second virus disease of the leatherjacket, *Tipula paludosa*. *Nature* 174 : 562-563.